FOF22F 6h9E200F

Figure 1

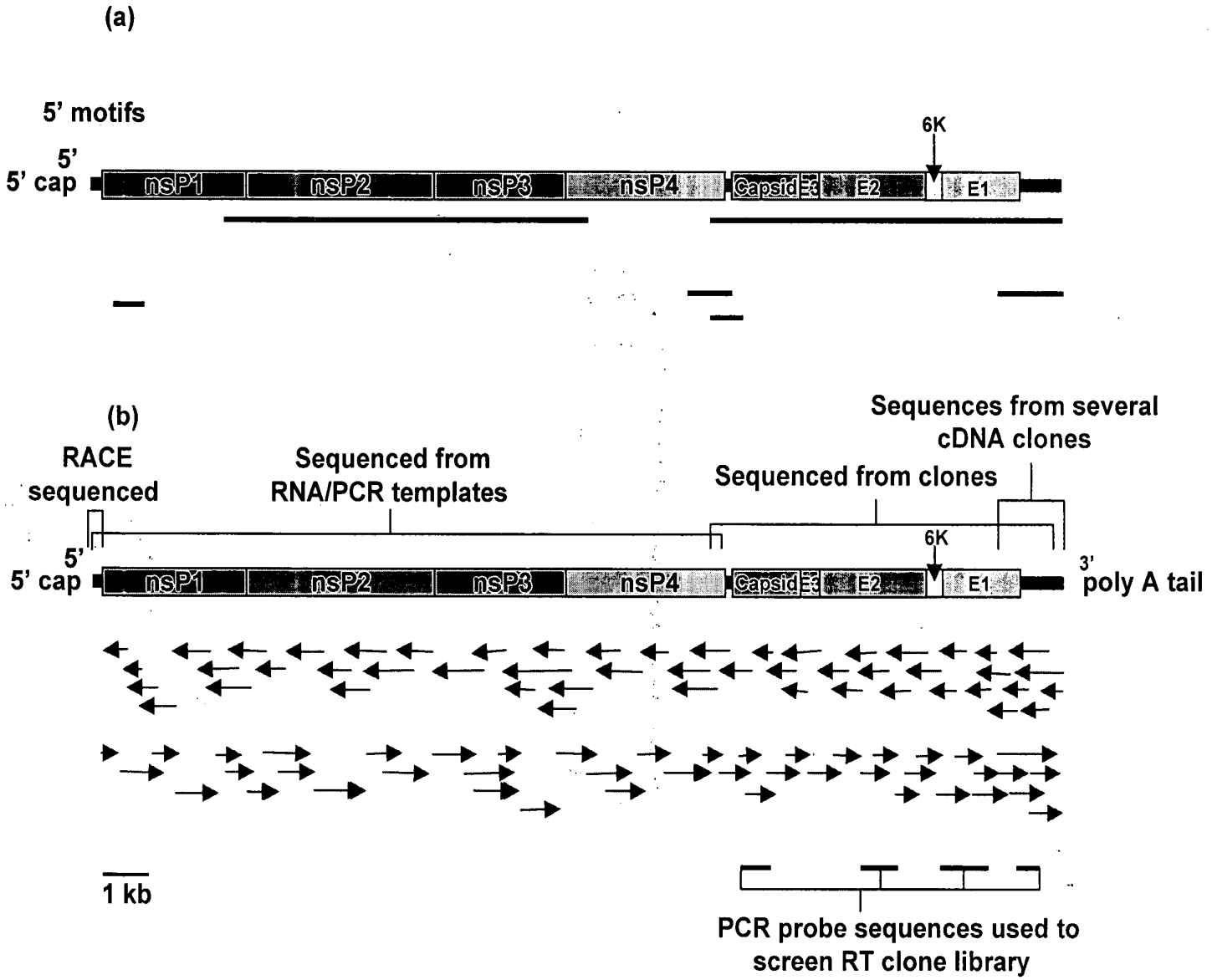
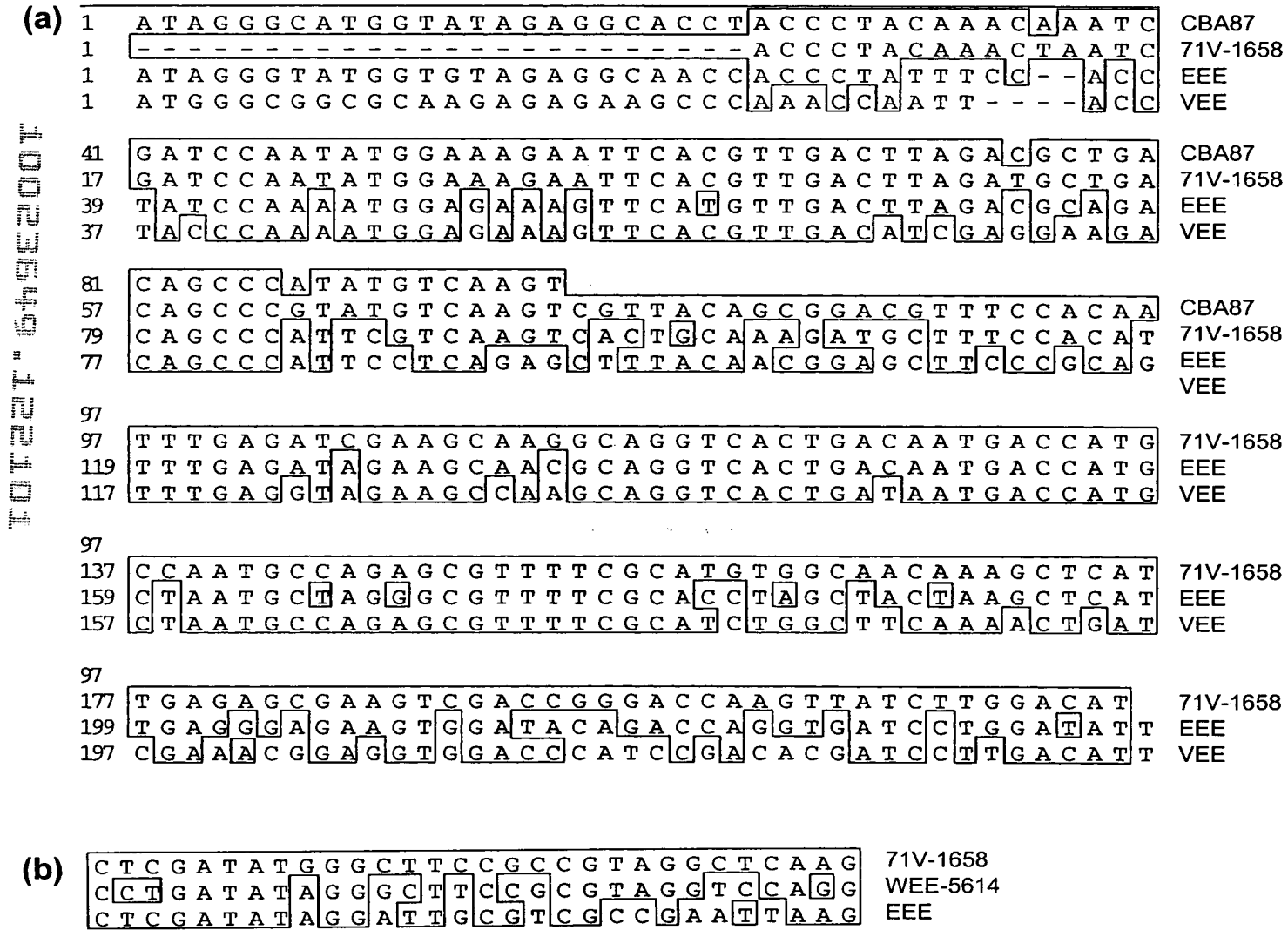


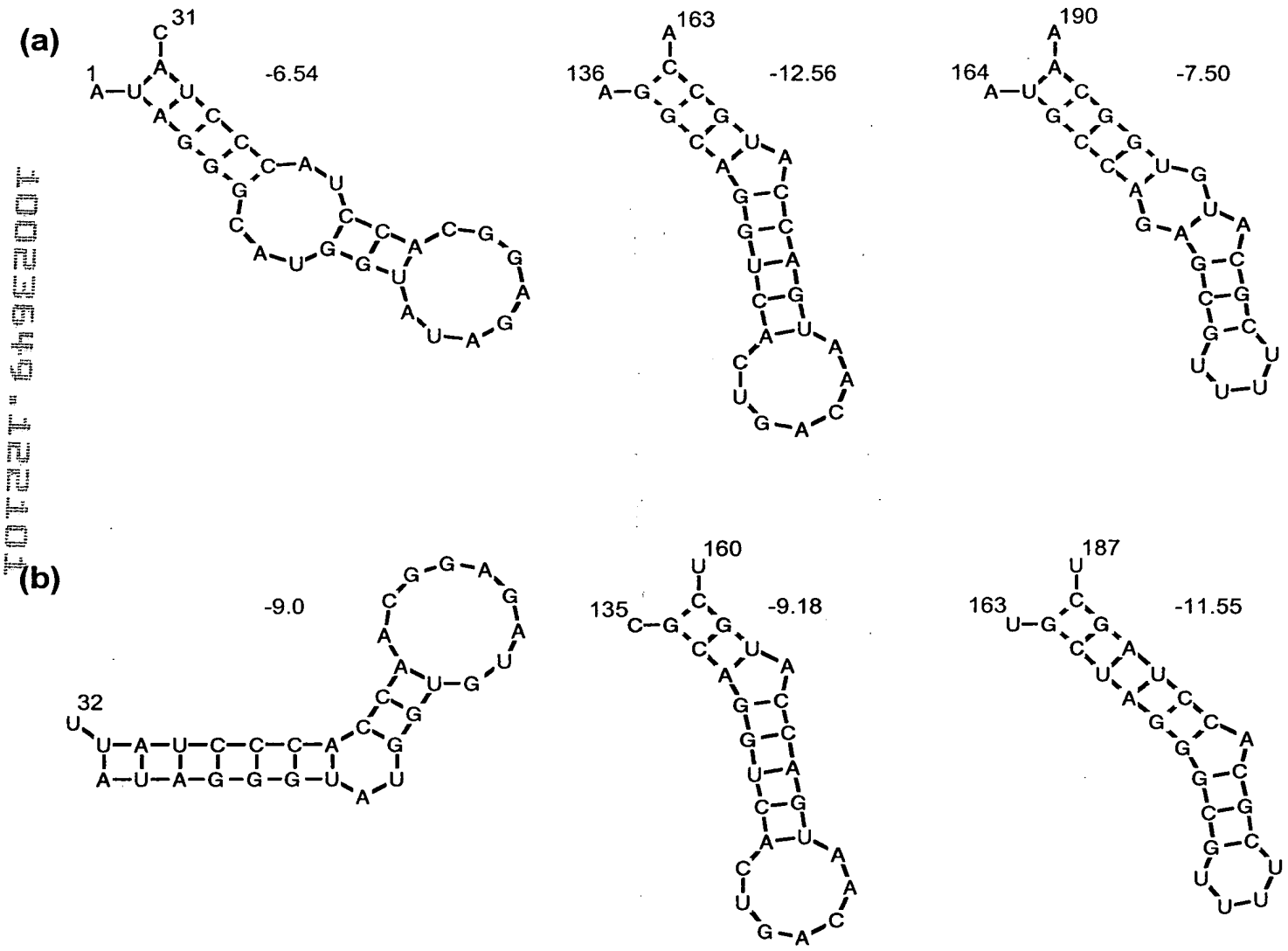
Figure 2 Multiple sequence alignment



a. The 5' terminus of WEE CBA87 (1-97), WEE 71V-1658 (25-240), EEE (1-238) and VEE (1-236) via Clustal module of DNAStar. Areas where sequences differ are boxed.

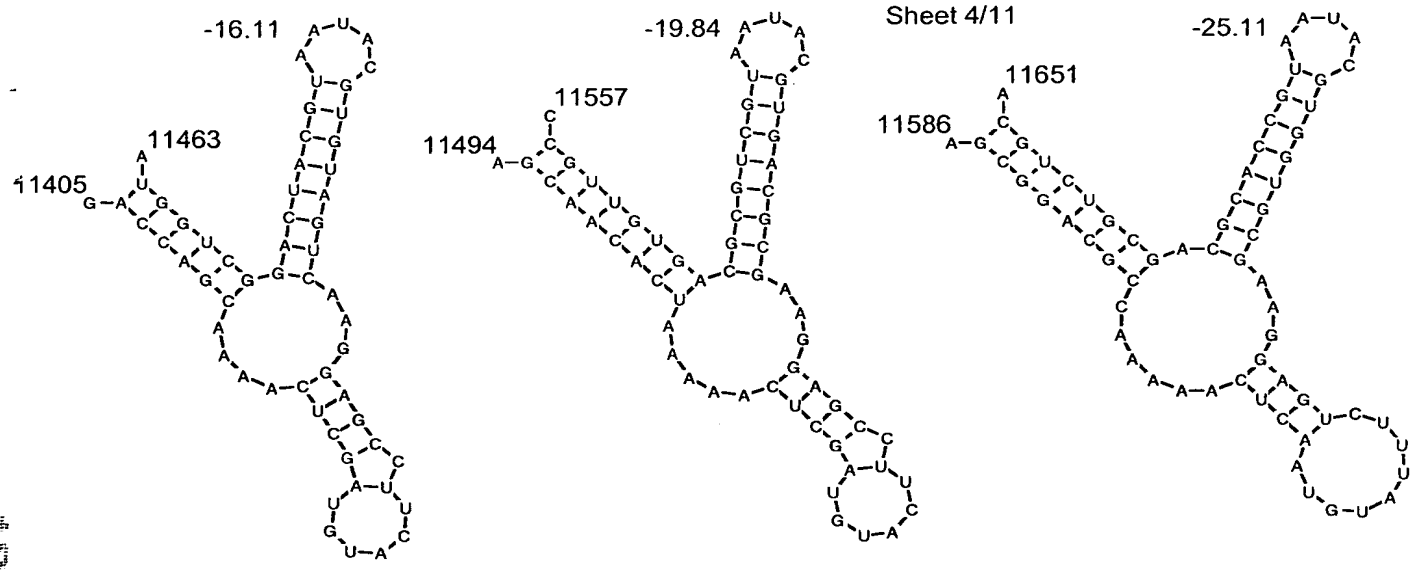
b. Hypervariable region identified in nsP1. Alignment of WEE 71V-1658 (1420-1449), WEE 1654 (65-94) and EEE (1415-1444) is shown.

Figure 3 Stem loop structures in the 5' NTR

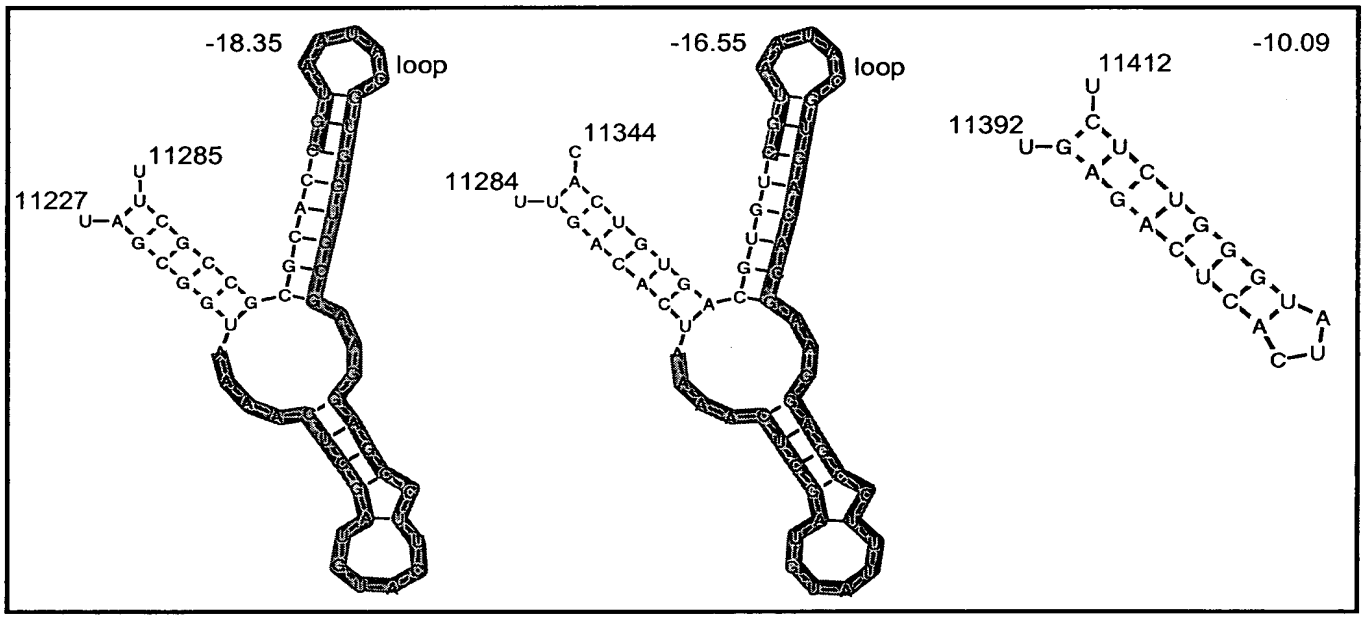


Hairpin structures were identified using the RNA folding program of the Genequest module (DNASTAR).

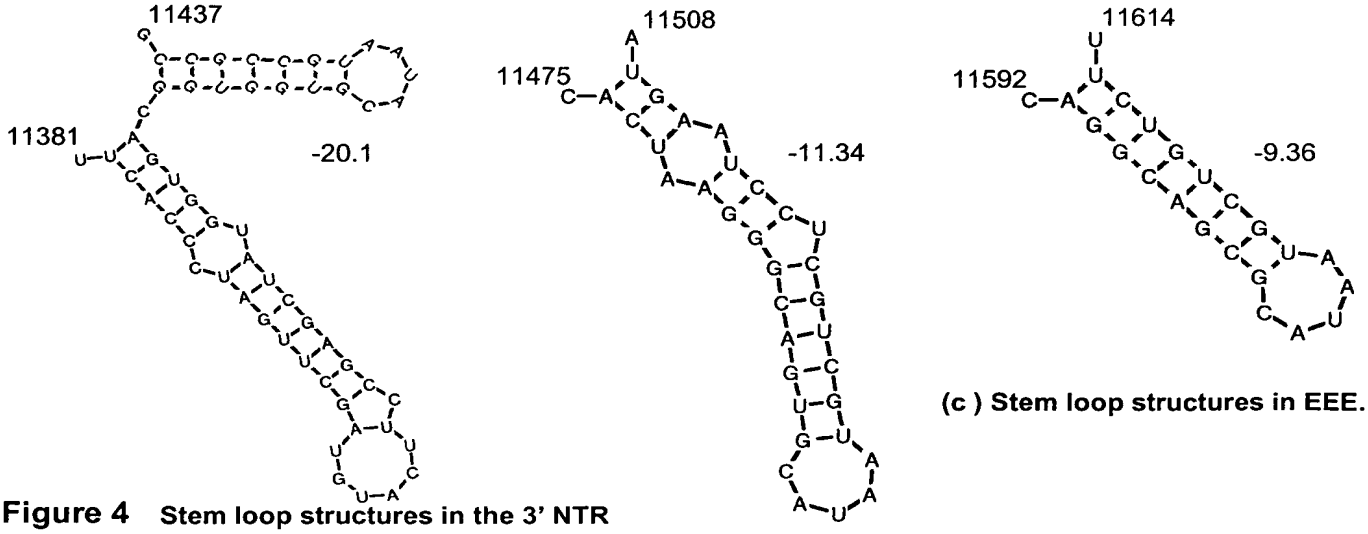
a. Structures for WEE (CBA87/71V-1658) sequence (1-192).
b. Structures for EEE (1-192).
Minimal free energy values are shown for the different structures.



(a) **Double stem loop structures in SIN.**



(b) Double stem loop structures in 3' NTR of WEE. Residues in the SIN-like 40 nt repeat are shaded.



(c) Stem loop structures in EEE.

Figure 4 **Stem loop structures in the 3' NTR**

Figure 5 **Phylogenetic relationship of the WEE nonstructural region compared to other alphaviruses**

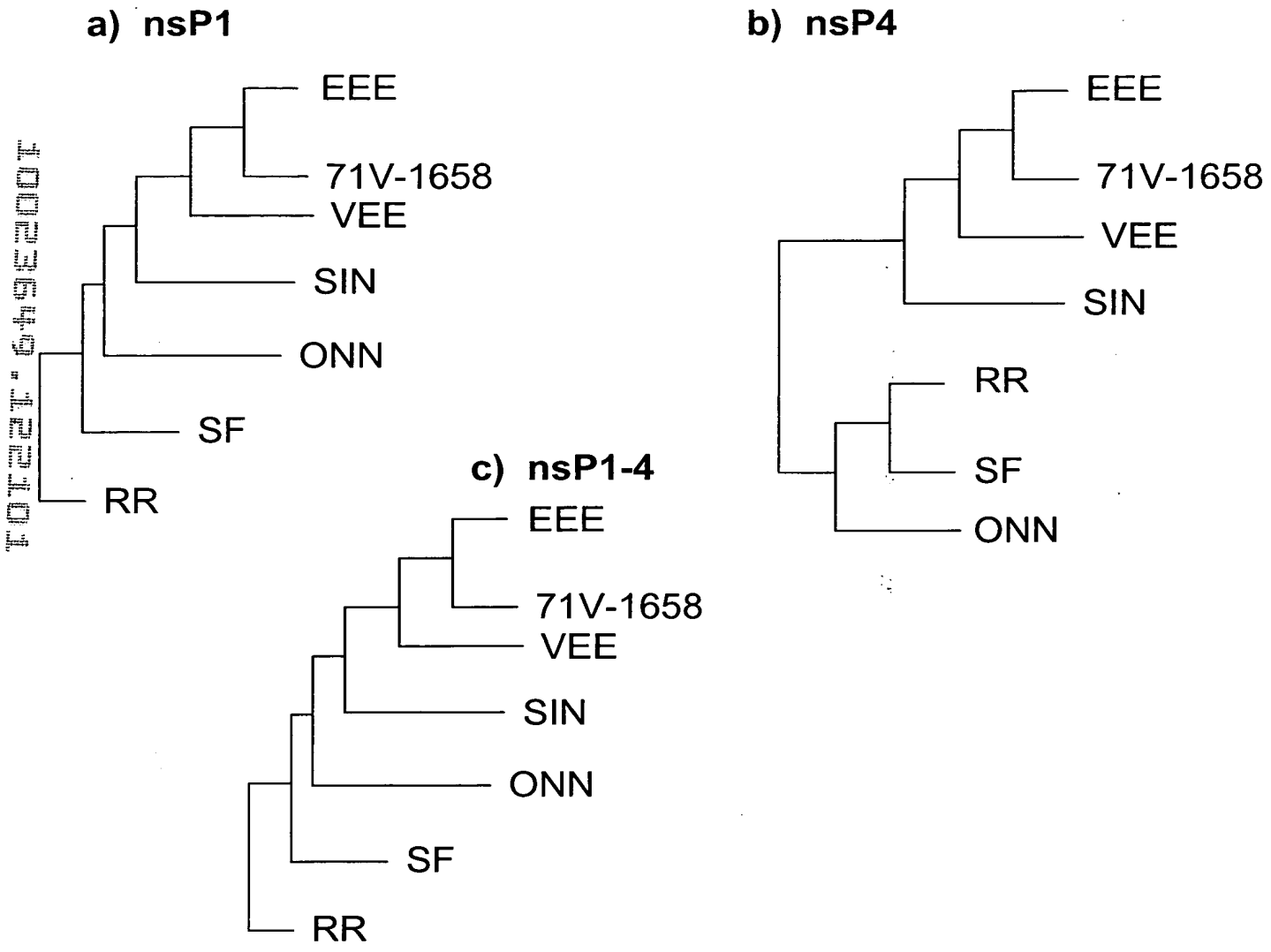
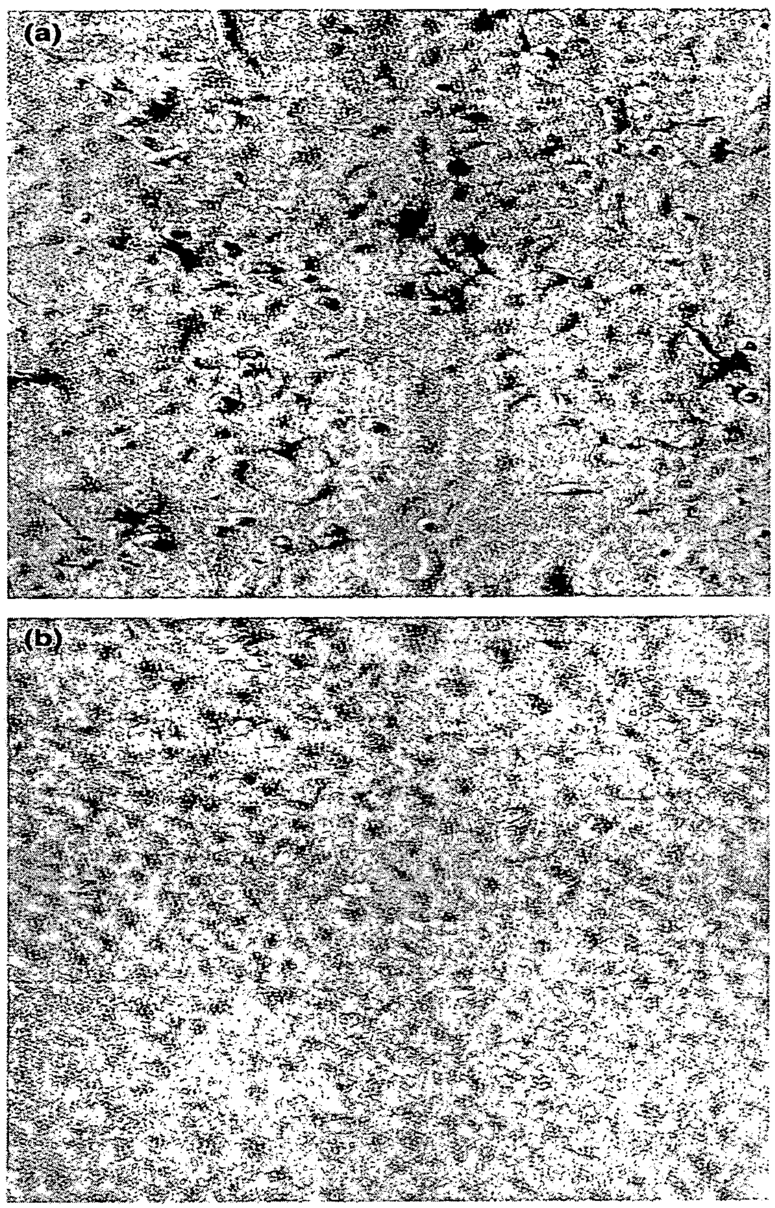


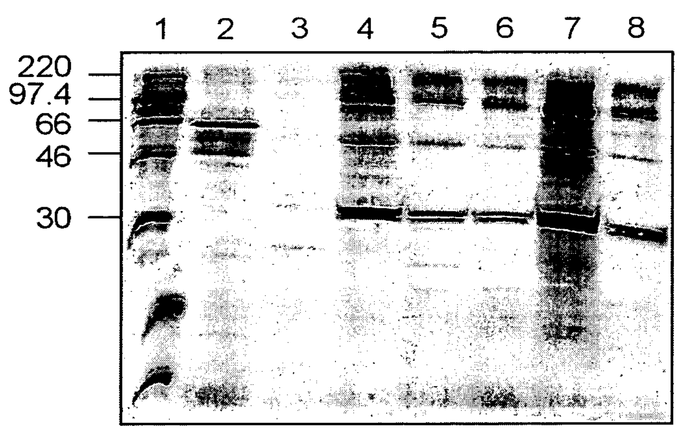
Figure 6 Expression of WEE structural genes in cell culture



One μg of plasmid DNA was transfected into Vero cells. After 31 hrs incubation, the cells were histochemically stained using a monoclonal antibody to WEE (11D2).
a. pCXH-3; b. pCI (control plasmid).

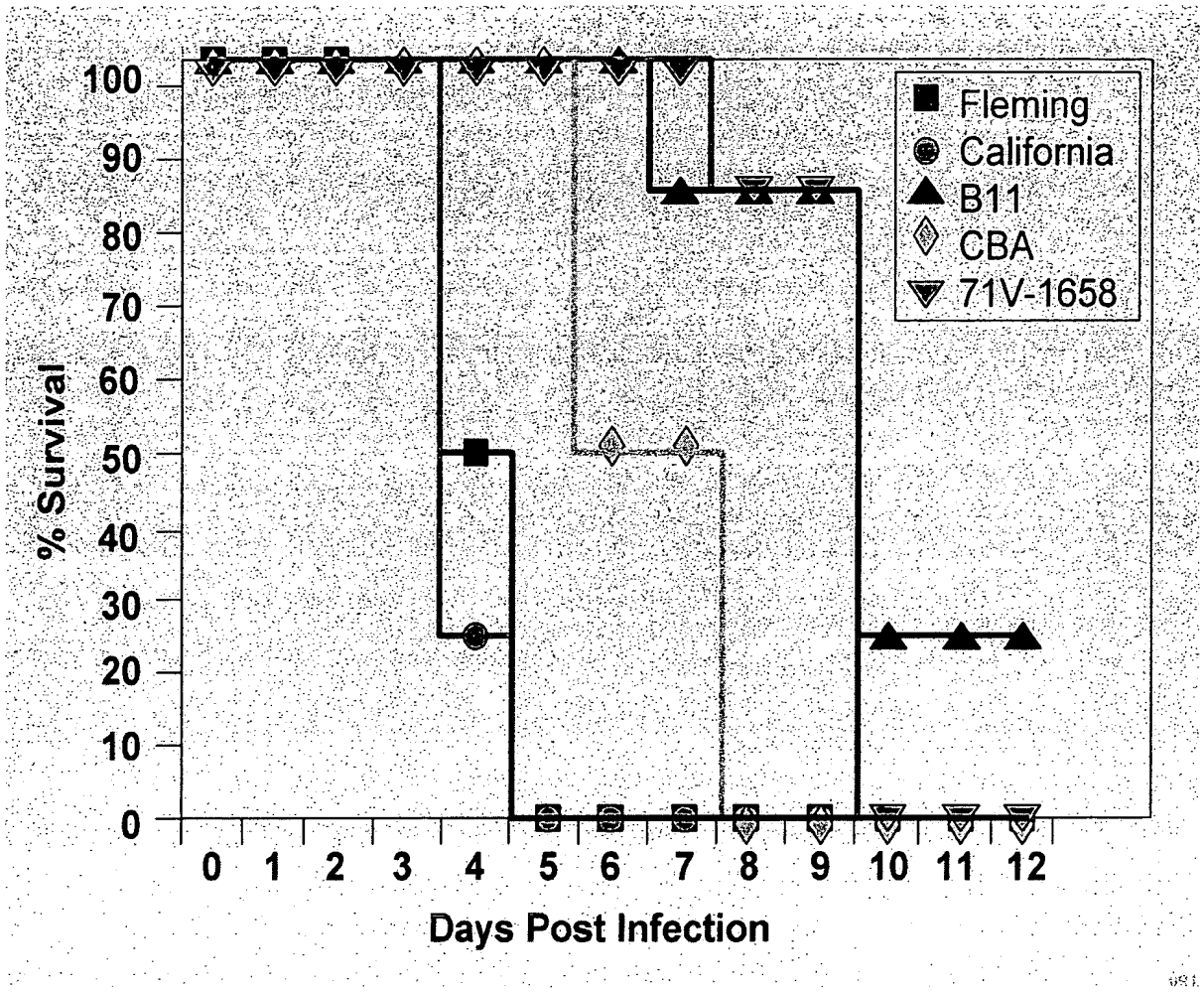
10023649.122101

Figure 7 In vitro transcription and translation of WEE expression vectors



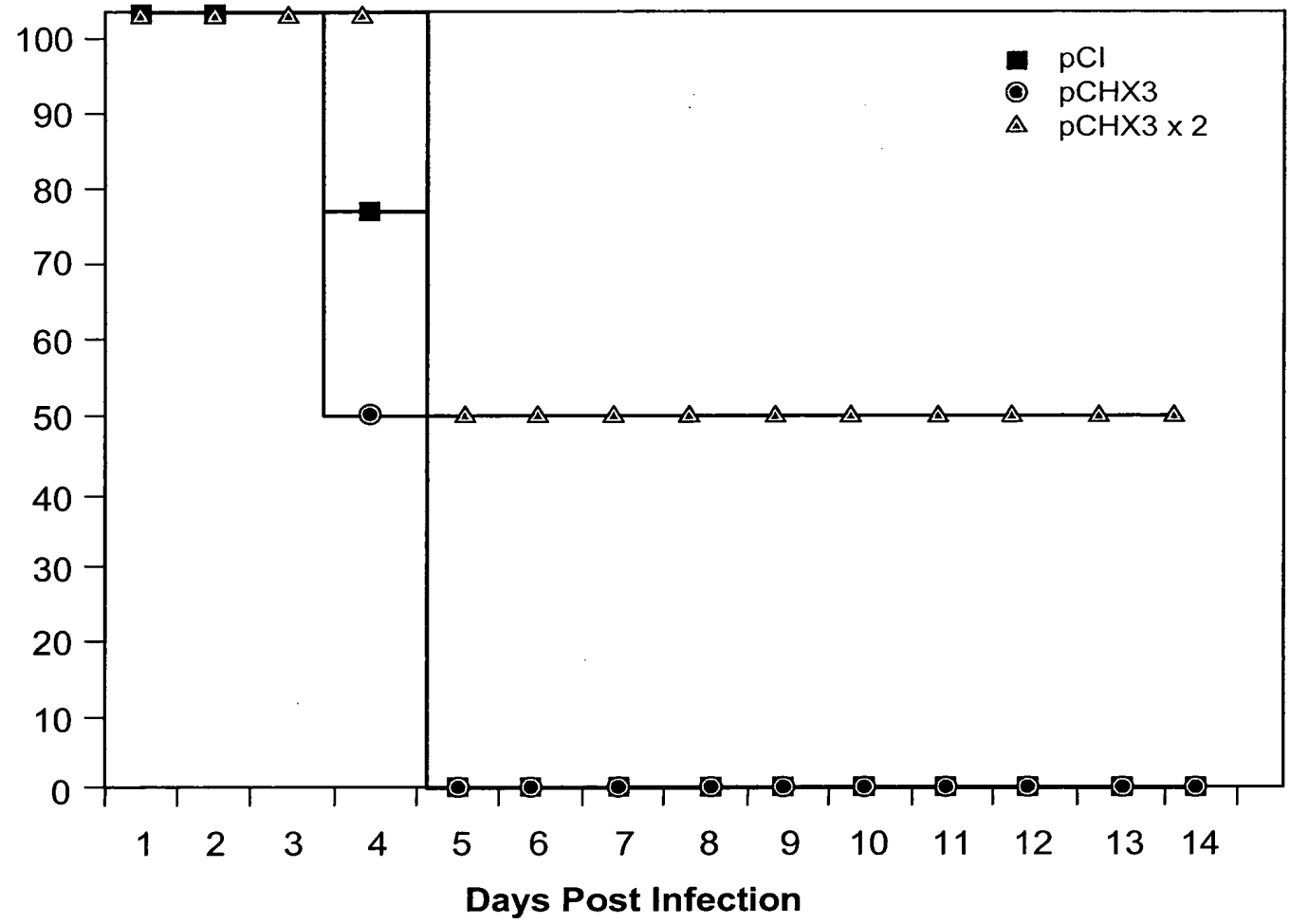
Qiagen purified vectors containing the WEE 26S insert were expressed *in vitro* using the TNT system and [^{35}S]-methionine labelling. Three μL aliquots of each samples were run by SDS-PAGE on a 12% gel.
Lane: 1) Rainbow ^{14}C-labelled marker; 2) Luciferase translation control; 3) pVAX; 4) pVHX-6; 5)pCXH-3; 6) pcDWXH-7; 7) pcDWHX-45; 8) pXTR2-4.

Figure 8 WEE mouse infectivity model



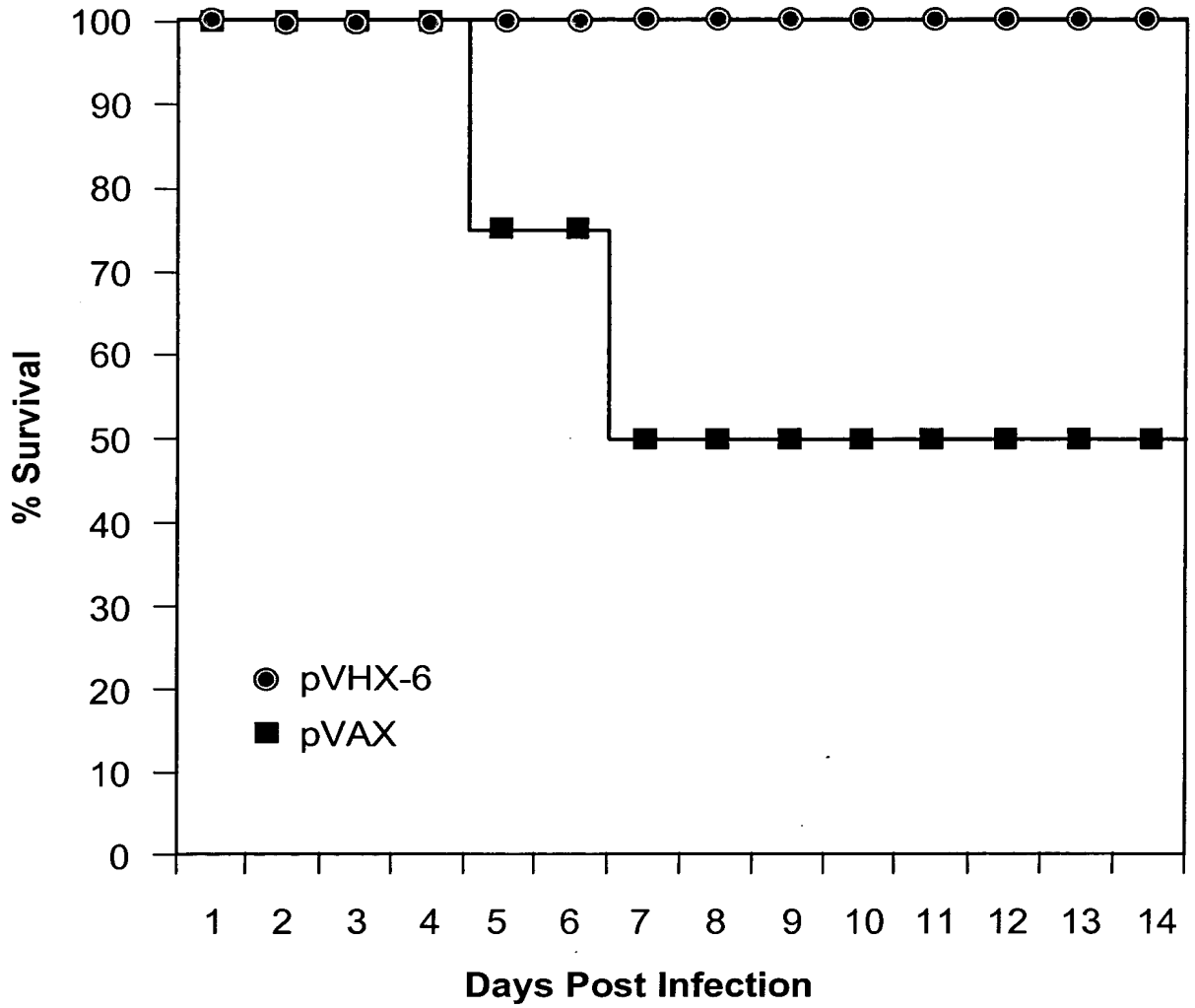
Groups of 4 mice were inoculated intranasally with 50 μL of virus (approximately 10^4 PFU). The mice were monitored for 12 days, and the % survival graphed.

Figure 9 Protection using ballistic delivery of pCXH-3



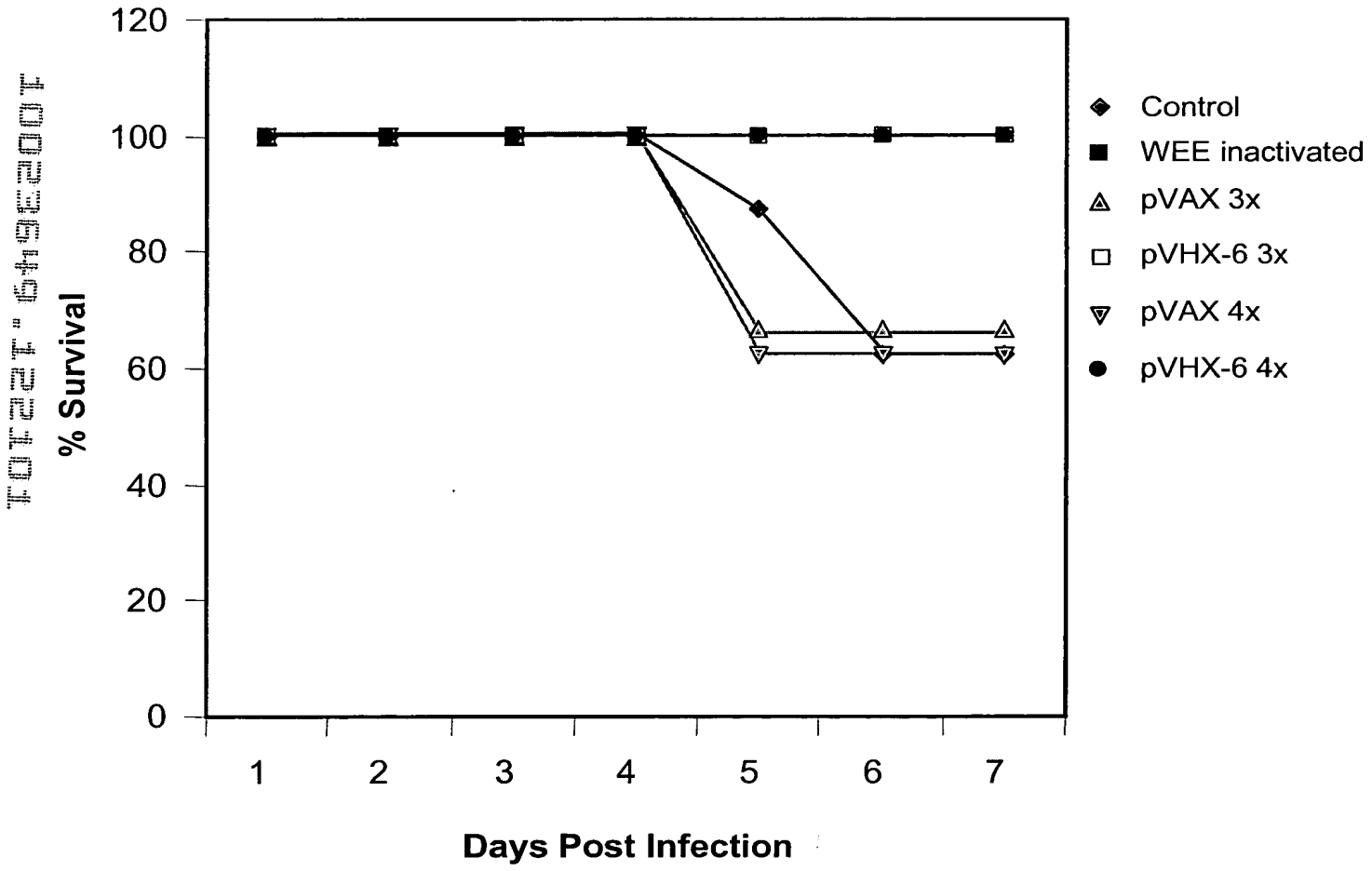
Groups of 4 mice were immunized with one or two doses (2 x 1.25 μg) of either pCI or pCXH-3. The interval between boosters (2 doses) or challenge was 3 weeks. The mice were challenged intranasally with 50 μL of WEE Fleming (1.25×10^4 PFU). The mice were monitored for 12 days, and the % survival graphed.

Figure 10 Protection using ballistic delivery of pVHX-6



Groups of 4 mice were immunized with four doses (2 x 1.25 μg) of pVAX or pVXH-6 . The interval between boosters or challenge was 2 weeks. The mice were challenged intranasally with 50 μL of WEE Fleming (1.25×10^4 PFU). The mice were monitored for 14 days, and the % survival graphed.

Figure 11 Protection using ballistic delivery of pVHX-6



Groups of 5-8 mice were immunized with three or four doses (2 x 1.25 μg) of pVAX or pVXH-6 . The interval between boosters or challenge was 2 weeks. The mice were challenged intranasally with 50 μL of WEE Fleming (1.7 x 104 PFU). Untreated control and WEE inactivated control (3 doses) groups were also included. The mice were monitored for 14 days, and the % survival graphed.